The role of the medical librarian in the basic biological sciences: a case study in virology and evolution

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DOI: 10.3163/1536-5050.96.4.004

INTRODUCTION

This current installment of the case study column concerns the role of the medical librarian in basic biological research. In the modern life sciences, the boundaries between the health professions and general biology are becoming increasingly blurred, as both disciplines rely to a greater extent on interdisciplinary, integrative, and comparative approaches for the resolution of major questions (Figure 1). Correspondingly, the university medical or health center library is rapidly becoming a primary resource for both basic and applied scientists from across virtually all fields of modern biology (e.g., from molecular and cellular biology to physiology to ecology and evolution). In response, the importance of the medical librarian as an information specialist continues to grow at these institutions. This case study documents this expanding role of the medical librarian by focusing on a basic research question that originated from a recent publication on viral evolution. The authors and editors invite your commentary on this case online at http://www.jmlacasestudies.blogspot.com.

THE CASE

JC virus (JCV) is a common virus in humans, its natural host* [1]. (Terms asterisked upon first appearance are defined in Table 1.) This virus was first isolated in 1971 from an immunocompromised* patient with the initials ''J. C.'' [2]. It is estimated that by age 20, nearly 90% of humans worldwide are infected with this virus based on the presence of JCV-specific antibodies [3]. Normally, JCV is not associated with any health problems, except in immunocompromised patients. In such patients, JCV infection can lead to progressive multifocal leukoencephalopathy (PML*), a fatal neurological disease [4].

A virus depends on its host for its survival and reproduction [5, 6]. Conversely, to the host, a virus represents a foreign element, which may lower its fitness*. Thus, viral evolution should mirror host evolution (and vice versa), because the survival and reproduction of the two are intertwined. In more formal terms, the expectation is for the virus and host to coevolve over time.

Working from this premise of coevolution*, Kitchen et al. [7] recently used DNA sequence data to compare the evolution and history of JCV to those of its human host. In contrast to almost all previous studies of the virus, these authors concluded that JCV was rapidly evolving on a timescale of decades to centuries, rather than tens of thousands of years [8–10]. Thus, their study indicated that the contemporary regional patterns of JCV diversity were due to a recent, rather than ancient, coevolutionary history with humans.

The second author of Kitchen et al. [7] is also the second author of this current case study (Miyamoto). One reviewer of the original manuscript [7] questioned whether JCV integrates into the chromosomes of its human host during its normal life cycle. Specifically, this reviewer wrote: ''from what I have read, some fraction of the time virus integrates and then replicates like a cellular gene …. On this basis, is it possible that there are two mutation rates, one reflecting cellular replication and one reflecting viral replication? Presumably, the former rate would be slower.'' In some types of viruses, viral DNA is integrated into the host's chromosome, and as such, the viral DNA becomes physically connected to the host's DNA. As recognized by this reviewer, the question of viral integration* is an important one, because an integrated JCV would be expected to evolve at the same (slow) rate as that for its host chromosome DNA, to which it would be physically linked. Thus, evidence of viral integration would oppose Kitchen et al.'s conclusion of a fast JCV rate [7]. In response to this reviewer, Kitchen et al. conducted a literature search to assess whether JCV integration is part of its normal life cycle. Based on this search, these authors reported in their final publication that ''JCV replicates in the host cell nucleus, but does not integrate into the latter's chromosomes'' [11].

Importantly, Khalili et al. [11] did not provide a definitive ''yes or no'' answer to the question of JCV integration. Nor did their article review the primary experimental evidence or citations in favor of or against this possibility. Instead, their article presented a basic introduction to the life cycle of JCV, which made no reference to viral integration. In the absence of a statement by them to the contrary, Kitchen et al. [7] cited their paper as at least implicit support for the absence of JCV integration during its normal life cycle.

However, as scientists, the three authors in general and one biologist in particular (Miyamoto) remain interested in whether hard experimental evidence exists in the primary literature for an explicit resolution of this problem. The biologist approaches you as an information specialist and asks for your aid in thoroughly searching this literature.

THE QUESTION POSED TO YOU AS INFORMATION SPECIALIST

Is there explicit evidence to indicate whether JCV does or does not integrate into the chromosomes of its human host during its normal life cycle?

Figure 1
Evolutionary biologist (Miyamoto) commentary

This case study highlights the importance of the medical librarian, as an information specialist, to the scholarly activities of the basic life scientist. As documented in this case, the expert searches and reviews of the medical librarian can greatly increase a basic life scientist's certainty that he now has a complete and accurate record of the primary literature in his area of interest. This record is critical for a full and thorough understanding of the main concepts, advances, and questions in his particular discipline. Such contributions of expertise by the medical librarian are necessary, given the interdisciplinary, integrative, and comparative nature of modern biology.

Conversely, the basic life sciences continue to make important contributions to the applied and scholarly activities of those in the health professions. As an evolutionary biologist, I compare DNA and protein sequences among different individuals and species to investigate their population genetics*, molecular evolution*, and phylogenetic relationships. Such approaches in evolutionary biology are becoming increasingly important to all disciplines of the life sciences, including those in the health professions [35]. For example, the origins and spread of new viral and bacterial pathogens and their increasing numbers of drug-resistant strains are two health-related areas that continue to benefit from the concepts and advances of evolutionary biology.

A particularly dramatic example of how evolutionary biology can benefit the health professions and their related fields comes from a criminal case in Louisiana, in which a gastroenterologist was accused of attempted second-degree murder [36]. The gastroenterologist was accused of deliberately injecting his ex-girlfriend with an HIV* sample, taken from an infected patient under his care, as a means of revenge. A team of evolutionary biologists (phylogeneticists) sequenced the same gene region from the HIV samples taken from the ex-girlfriend, the patient under the care of the gastroenterologist, and other infected patients in the local general population. An evolutionary tree (phylogeny*) was estimated for these HIV samples from their patterns of gene sequence similarity and dissimilarity. The resulting phylogeny clearly demonstrated that the HIV sample from the ex-girlfriend was closely related to that of the patient under the care of the gastroenterologist. This direct connection between the victim and gastroenterologist's patient provided important evidence for a successful conviction of the physician for attempted second-degree murder.

Evolutionary biology in particular and the basic life sciences in general continue to enrich the health professions and their investigations of epidemics and pandemics, emerging new diseases, drug resistance, and the like [35]. The court case highlighted above—combining evolutionary biology, forensics, and the criminal justice system—serves as a dramatic reminder of this interdisciplinary importance among the applied, basic, and clinical disciplines of the health professions. Other examples of the use of evolution and phylogenetics to solve biomedical and other biological questions can be found in the module "Phylogenetics Resources" [37], part of the National Center for Biotechnology Information's (NCBI's) "Advanced Workshop for Bioinformatics Support Specialists" [38].

Furthermore, librarians at university health sciences libraries are increasingly finding themselves as information providers for nonmedical biologists, whether their research programs inform the health professions or not. As more basic biology investigations (zoology, ecology, conservation, animal behavior) shift to molecular and genetic approaches, more nonmedical research biologists find their information resources and expertise in health sciences libraries. For example, at the University of Florida, zoologists are using molecular techniques to examine a gamut of research questions, many of which require the use of resources under the auspices of the health sciences library: molecular development of limbs and genitalia; genetic variation, gene expression, and mutation in *Drosophila* and *Caenorhabditis*; molecular aspects behind pair-bonding and communication in the prairie vole and singing mouse, respectively; effects of environmental contaminants (particularly endocrine disruptors) on wildlife development and reproduction; and genome evolution in birds and alligators. Anthropology (the peopling of the Americas using molecular evidence, the genetics of alcoholism); botany (floral genomics and phylogenetics); and agriculture (horticultural sciences, plant pathology, microbiology and cell science) researchers all use, to some extent, many of the same resources as biomedical researchers employed by the medical school. Although these nonmedical researchers may have a home library dedicated to science elsewhere on campus, because molecular and genetic resources are often housed at the campus health sciences library, medical and health sciences librarians can expect to encounter increasing numbers of nonmedical researchers with information needs that fall under the purview of the health sciences library. This movement to molecular approaches provides an exciting opportunity for health sciences librarians to extend the application of their expertise beyond the walls of the health sciences center.

UNDERSTANDING THE CONCEPTS

Your initial discussions with the biologist identify more completely the concepts that Kitchen et al. [7] are specifically concerned with: whether JCV *normally* integrates into the chromosomes of its *natural, permissive* human host.* Thus, your first job is to become more familiar with JCV and terminology related to virology (such as "permissive"). You must understand a number of basic concepts to perform a search that might retrieve information on this topic. Because you are not necessarily conversant in virology, starting with a basic virology textbook can provide insights into these concepts. Two commonly used textbooks include Wagner and Hewlett's *Basic Virology* [12], which is often used in beginning virology classes, and *Fields' Virology* [13], which is a standard and more advanced virology reference work. While either work will define basic concepts, the Wagner and Hewlett work is more accessible to someone new to virology.

Both works indicate that the human JCV is a member of the polyomavirus* family (Polyomaviridae), a group of viruses that have DNA as their genetic material (in other words, they are DNA viruses). Other members of Polyomaviridae include simian virus 40 (SV40*) and the BK virus (BKV). A quick search in PubMed (JCV OR "jc virus") retrieves over 1,400 papers, more than you or the biologist deem appropriate to look at individually. Over 10 times as many papers (over 17,000) are retrieved from PubMed for the closely related SV40 (SV40 OR "simian virus 40"), confirming that it is a well-studied virus. A search on the less well-known BKV ("BK virus" OR BKV) retrieves over 1,200 articles. From this cursory glance, it appears much more is understood about the basic biology of SV40 than of either JCV or BKV, so it is possible that if information is not available for JCV, it could be reasonable to use SV40 as a model to explain JCV. Further reading indicates that JCV had initially been assigned to the family Papovaviridae, so this term may become important if it is necessary to search for information published on JCV or other members of the family prior to the taxonomic reassignment [14].

A key aspect of the biologist's question is whether or not JCV normally integrates into permissive cells, so you must understand this concept as well. According to the textbooks you have used, "permissive" cells are those in which a virus can grow and replicate, while a nonpermissive* cell is one a virus may enter and potentially begin growth in but not complete it. As stated on page 132 of *Basic Virology*, a nonpermissive cell is one that "for some reason, does not have the proper machinery for virus replication" [12]. According to the research accumulated by the authors of the Kitchen et al. paper [7], it has been well established that JCV may integrate into the chromosomes of other mammalian species (e.g., rodents and monkeys) [15, 16]. In these cases, JCV integration is associated with the formation of tumors in these foreign* nonpermissive hosts. Along these lines, JCV has also been associated with certain human tumors, although whether this relationship is one of cause-and-effect (as in the other species) remains unresolved [17, 18]. So although these papers demonstrate JCV integration in abnormal circumstances in nonpermissive cells, they do not provide experimental evidence concerning the normal, permissive, human condition required of the question at hand.

Another distinction provided by the biologist concerns the location of potential JCV integration. If integration normally occurs, does it occur in the kidneys of infected adult and juvenile humans, as opposed to other organs or tissues? This distinction is important, because it is widely accepted that JCV is transferred among humans through urine [1]. Thus, the kidneys have been shown to function as the ''reproductive organ'' for JCV transmission, thereby making them the primary focus of the coevolutionary study by Kitchen et al. [7].

Despite these two distinctions, the biologist makes the final important point that every study about JCV integration may potentially be important, including those that are not specifically concerned with the life cycle of the virus in the kidneys of healthy juvenile and adult humans. In the absence of direct evidence obtained under ideal conditions, knowledge about viral integration in diseased patients, other species, and different organs may still provide sufficient evidence to support (rather than confirm) the hypothesis of no JCV integration in the kidneys of healthy humans. You keep all of this in mind as you outline your search and review strategies.

BUILDING THE SEARCH AND EXPLORING THE LITERATURE

Before you begin serious work on your literature search, you peruse both textbooks once more for any additional clues concerning potential JCV integration. You first note that, on page 2285, *Fields' Virology* [13] states in relation to viral latency* and reactivation*: ''Whether JCV DNA integrates into the cellular DNA or remains episomal* is not known'' [1]. An earlier statement in the text, on page 2281, reiterates this notion for the polyomaviruses: ''No information is available, however, on the mechanisms of viral persistence*, whether the viral DNA is episomal or integrated, what triggers viral synthesis and multiplication '' [1]. Later in the same paragraph, it is stated for polyomaviruses in general that ''viral latency can be established in which only the viral DNA is present without evidence of viral protein synthesis*. In this circumstance, as with the herpes family of viruses, viral DNA may be integrated into the cell chromosome or remain episomal and then reactivated under various host events.'' However, the discussion does not specifically state that this has been observed in JCV, and no references are cited for this statement, a requirement necessary for solving the question at hand.

Figure 17.2 in *Basic Virology* [12], illustrating the replication cycle of the closely related SV40 in a permissive cell, shows no viral integration but provides no references to associated experimental evidence. A statement later in the text, on page 296, in relation to SV40 replication in nonpermissive cells describes the integration of SV40 DNA into the host genome, noting that it is ''entirely random'' and ''does not occur very frequently'' [12], but when it does happen, it can cause cell transformation and cancer. Thus, this aspect of the biology of SV40, JCV's relative, parallels the literature retrieved by Kitchen et al. [7] concerning lack of normal integration by JCV.

You make one last attempt to answer the question conclusively before you resort to a refined literature search. You refer to *Virus Taxonomy: Classification and Nomenclature of Viruses*, a text on the taxonomy of viruses. Because this resource reflects the work of the international committee that names and classifies viruses, it can be considered an authoritative text on viral taxonomy. According to this work, when charged with building and maintaining the virus taxonomy, the ''goal of this undertaking is to categorize the multitude of known viruses into a single classification scheme that reflects their evolutionary relationships, i.e. their individual phylogenies'' [19]. Such phylogenies usually are based on shared biological characteristics, and you are hopeful that in its discussion of the group, the text will discuss the integration issue for JCV or one of its close relatives. However, no discussion of integration is available except for the following sentence on page 236: ''In transformed* and tumor cells, the polyomavirus genomes are usually integrated into chromosomes of the host cell'' [19], a fact that you already know. On page 6 [19], the text refers to ''viruses that can integrate into the genome of the host, such as retroviruses and lysogenic bacteriophages*,'' two groups that are not closely related to the Polyomaviridae. These statements suggest that members of the Polyomaviridae do not integrate under normal conditions in permissive cells, however, no experimental evidence is provided.

Before you begin a literature search, you take this information back to the biologist to be sure that it is not sufficient to answer his question. He agrees that the information that you have found using the texts does not provide conclusive experimental evidence, and as such, his question still requires a literature search. You now start your exploration of the primary literature by searching PubMed.

By using the Medical Subject Headings (MeSH) database, you learn that a MeSH term exists for the virus of interest, ''JC Virus,'' and that this term was used from 1983–1993 and again since 2002. Previous indexing (1971–1982) had placed JCV under the family to which the virus was formerly assigned (Papovaviridae) and the common name of the group to which it is now assigned (polyomaviruses); however, you cannot tell from the online MeSH database what the MeSH term was from 1994–2001. You refer to the print Annotated Alphabetic List from 1994 and see that the MeSH term is ''Polyomavirus Hominis 2.'' From your reading, you know that JCV is currently a member of the family Polyomaviridae, but, as MeSH suggests, it was reassigned to that family from the Papovaviridae in 2000 [14]. The MeSH database provides you with a number of alternative entry terms that may be useful in searching PubMed or other databases: human polyomavirus JC, JC polyoma virus, polyomavirus hominis 2. Through reading the *Fields' Virology* entry on JCV [1], you know that the virus was isolated in 1971 by Padgett et al. from a patient with PML [2]. When you retrieve a copy of the Padgett paper, you see that the virus is not referred to as the ''J.C. virus'' until late in the paper; instead, in the title and summary it is referred to as a '''papova-like' virus associated with progressive multifocal leucoencephalopathy.'' Based on all the information gained from these resources, you have a number of terms that you may use for JCV, and you perform a more complete search than you did when you started your investigation:

(jc virus [mesh] OR ''papova-like'' [tw] OR jcv [tw] OR ''jc virus'' [tw] OR ''human polyomavirus JC'' [tw] OR ''JC polyoma virus'' [tw] OR polyomavirus hominis 2 [mesh] OR ''polyomavirus hominis 2'' [tw] OR ''J C Virus'' [tw])

You retrieve over 1,400 articles, including a few articles not retrieved by your quick introductory search.

You must now address the ''integration'' aspect of the search. MeSH provides the term ''Virus Integration,'' the ''insertion of viral DNA into host-cell DNA.'' This term was introduced only in 1992, and, in earlier years, very broad terms were applied to such papers (''DNA, Viral'' and ''Proviruses''). These terms are so broad in their scope that you decide to introduce the integration aspect in another manner: a search using ''integrat*,'' ''nonintegrat*,'' and ''non-integrat*'' as textwords. Therefore, your search statement reads:

(jc virus [mesh] OR ''papova-like'' [tw] OR jcv [tw] OR ''human polyomavirus jc'' [tw] OR ''jc polyoma virus'' [tw] OR polyomavirus hominis 2 [mesh] OR ''polyomavirus hominis 2'' [tw] OR ''jc virus'' [tw] OR ''j c virus'' [tw]) AND (virus integration [mesh] OR integrat* [tw] OR non-integrat* [tw] OR nonintegrat* [tw])

This search retrieves about 20 citations.

A look through this list of articles reveals that most of them do not meet the criteria of ''normal, human, permissive'' cells. Retrieved articles cover the gamut of those involving JCV related to carcinomas or other cancers to schizophrenia, as well as in species that are not normal hosts for JCV, including owl monkeys and hamsters [15, 20–22]. Although most of the articles look like they could immediately be eliminated, you remember that the biologist cautioned that in the absence of other evidence, it is possible that some articles that are peripheral to your topic may need to be considered. You review the abstracts and full text of several articles that deal with disease or nonpermissive hosts; none appear to be useful. You also discard those that overlap with the papers initially screened by Kitchen et al. [7], as these have already been thoroughly reviewed by the author team.

You notice that three papers in the set of twenty look particularly relevant. Grinnell et al. [23] discuss nonintegrated JCV DNA in patients with PML. Because this paper specifically notes ''nonintegrated'' DNA (albeit from diseased patients), it is possible that it might provide useful insights into the normal situation in JCV. A paper by Dorries and ter Meulen [24] also looks interesting, as it describes persistence in the kidney, although, again, in patients with PML. Finally, the title of the paper by Chesters et al. [25] mentions the persistence of JCV DNA in both normal individuals and PML patients, therefore it is reasonable to think that article may provide the most relevant information. A sentence in the abstract of the paper strikes you as especially relevant: ''The viral DNA detected appeared not to be integrated with host DNA and to be isolated in foci.'' You place this paper to the side for the biologist.

You note that none of the located papers were published before 1980, although JCV was first isolated in 1971 [2]. You decide to search for *any* papers on the virus published prior to 1980 (1971–1979) to see how the virus was being described during this initial period after its discovery and limit the search to ''humans'':

(''papova-like'' [tw] OR jcv [tw] OR ''jc virus'' [tw] OR ''human polyomavirus jc'' [tw] OR ''jc polymoma virus'' [tw] OR ''polyomavirus hominis 2'' [tw] OR ''j c virus'' [tw]) AND 1971:1979 [pdat] AND humans[mesh]

You now retrieve about 25 articles, but on examining titles and abstracts, none appear to be relevant to the question at hand, as none relate to infection in healthy humans or the other criteria expressed as important by the biologist.

You take a new direction and decide that limiting to ''integration'' may have excluded some articles that describe the general life cycle of JCV and that those papers might consider the integration question. You reformat your search to omit the integration terms and limit the search to ''review'' articles related to JCV in humans by adding ''AND review[pt]'' to your basic search string, retrieving about 200 review articles. As you scroll through the list, you note that 13 articles are all from 1 issue of *Advances in Experimental Medicine and Biology* [26]. When you examine the full text of this issue, you see that the volume title is ''Polyomaviruses and Human Diseases'' and that it contains a number of chapters concerning JCV. You set this volume aside for the biologist.

To make sure you have thoroughly explored any potentially relevant literature, you try one more strategy. You have not yet used the broader terminology of the genus or family names to which JCV currently belongs or its former taxonomic designation. Perhaps a useful paper has been indexed under these broader terms rather than specifically under JCV. You search:

Table 1
Key concepts for this case study

Key concept	Brief definition
Coevolution	Mutual heritable changes between two lineages over time such that new adaptations in one drive the origins of corresponding adaptive features in the other. In the case of a viral pathogen and its host, the latter constitutes the environment in which the former must survive and reproduce. Thus, the virus may evolve new amino acids in one of its structural proteins to avoid the defenses of the host immune system. Conversely, to the host, the virus represents an intracellular parasite, whose reproduction comes at its expense. In response, one or more of the host immune proteins may next acquire new amino acids for the further recognition and neutralization of the evolved virus. In this example, these mutual interactions may lead to an escalation of adaptations and counteradaptations (i.e., a coevolutionary arms race) between both the virus and host.
Episomal	Formally, a genetic element that can replicate as either a linked part of a host chromosome or as a free unlinked factor, independent of its host chromosome(s). However, following previous authors [e.g., 1, 27], this case study limits the use of the term episomal to only the latter state of a free unlinked factor. In this way, the authors distinguish between the 2 potential states for the polyomavirus DNA (i.e., unlinked [episomal] versus linked [integrated] to a host chromosome).
Evolutionary rate	In this case study, the frequency at which new mutations arise in the DNA sequence of a species over time. Specifically, Kitchen et al. [7] measured the JC virus (JCV) evolutionary rate in units of new mutations per base position of the viral DNA sequence per year.
Fitness	The relative success of an individual, genotype, and/or allele to survive and reproduce in its environment.
Foreign host	Species that is not the natural target for a virus and therefore usually (but not always) does not support a productive viral infection. Thus, a foreign host is typically also a nonpermissive one, because the virus is usually ill adapted to survive and reproduce in this unnatural setting. Rodents and monkeys represent foreign hosts for JCV, which are also nonpermissive.
HIV	Human immunodeficiency virus, which is the causal agent of the acquired immune deficiency syndrome (AIDS) in humans.
Hybridization with Southern blotting	Laboratory technique (named after its developer) for identifying a specific DNA sequence in a complex population of DNA fragments. Specifically, this population refers to a mixture of different DNA sequences after their separation by gel electrophoresis. Southern hybridization uses the DNA sequence of interest as a probe, which is first labeled (e.g., radioactively) and then added to the population of separated DNA fragments. The labeled probe is now allowed to bind, or hybridize, to copies of itself via complementary base pairing (i.e., A with T and C with G). In this way, the target DNA in the population becomes labeled too, thereby allowing the investigator to track its presence and linkage to other DNA.
Immunocompromised	Refers to a person with a defective immune system, who is thereby vulnerable to opportunistic infections and diseases that do not usually affect normal individuals. In the case of JCV, PML is one such condition.
Integrase	The enzymes of certain viruses (e.g., HIV) that direct normal insertion of their viral DNA into their host chromosome's DNA. The lack of an integrase gene in polyomaviruses was used by Lednicky and Butel [27] as the first of 2 lines of evidence for no normal integration by them into their host chromosomes.
Integration	The insertion of viral DNA into host chromosome DNA, thereby leading to a direct physical linkage between the 2. Although such integration by polyomaviruses is known to occur in foreign nonpermissive hosts; such insertions nevertheless are rare and are random in terms of their chromosome positions. This rarity and randomness were used by Lednicky and Butel [27] as the second of 2 lines of evidence against normal polyomavirus integration in their natural permissive hosts.
Latency	The longer-term persistence of a virus in its host, during which there is no protein synthesis or active reproduction by the former and no significant disruption of the cell cycle of the latter. During this period of dormancy, the viral chromosome remains intact, thereby allowing for future reactivation and growth of the virus.
Lysogenic bacteriophage	A bacteriophage is a virus that infects bacterial cells for its reproduction. A lysogenic (temperate) bacteriophage is one whose life cycle includes the integration of its viral chromosome into that of its host. During this lysogenic phase, the linked viral and host DNAs co-replicate together, as the host otherwise continues with its normal life cycle. At some later point, the viral chromosome is excised from the host DNA, thereby once again becoming an independent cytoplasmic factor. This excision marks the start of the subsequent lytic phase during which the virus reproduces and completes its own life cycle.
Molecular evolution	Interdisciplinary field of evolutionary biology and genetics, concerned with the patterns, processes, and consequences of change in the informational macromolecules (DNA, RNA, and proteins).
Natural host	Species that is the normal target for a virus in its native environment. As its normal target, the natural host is also a permissive one that supports a productive viral infection. Humans are the natural permissive host for JCV.
Nonpermissive host	Species or tissue that is not the normal natural target for a virus and therefore does not support a productive viral infection. Thus, such an infection is abortive in that the polyomavirus begins its life cycle but then fails to reproduce or does so inefficiently. On rare occasions, the viral DNA may randomly integrate into a nonpermissive host chromosome, thereby leading to a tumor. Such abortive infections by JCV can occur in rodents and monkeys, which are not its natural host species.
Permissive host	Species or tissue that allows for a productive viral infection (i.e., one where the virus can efficiently reproduce and complete its entire life cycle). Usually, but not always, a permissive host is also the natural normal target for the virus in the wild. In the case of JCV, humans are its natural host, as well as its only permissive one. In the case of SV40, African green monkeys allow for permissive growth, even though they are not its natural normal host in the wild (which instead are rhesus monkeys).
Persistence	The long-term infection of a host by a virus, during which the latter remains latent and/or reproductively active, depending on the tissue. In its human host, JCV persists in a reproductive state in the kidneys but in a latent one in the brain and gastrointestinal tract.
Phylogeny	The evolutionary history of a related group of populations and species or their genes and proteins. These histories are depicted as evolutionary trees, which summarize the branching order of ancestral splits into descendant lineages over time. In essence, then, phylogenies correspond to "family trees" or "pedigrees" for populations, species, genes, and/or proteins.
PML	Progressive multifocal leukoencephalopathy, which is a fatal demyelinating disorder of the central nervous system. PML was very rare until the start of the AIDS pandemic. It is caused by the reactivation of JCV in immunocompromised patients, thereby leading to the death of those cells (oligodendrocytes) that are responsible for supplying the central nervous system with its electrically insulating myelin.
Polyomavirus	The sole genus of the family Polyomaviridae, with 13 recognized species (including JCV and BKV of humans and SV40 of monkeys). Polyomaviruses are small viruses, with a genome consisting of a single, circular, double-stranded DNA molecule of ~5,000 base pairs. This genome encodes various genes for both regulatory and structural proteins, as well as a noncoding control region. Polyomaviruses can cause tumors in animals and in their tissue cultures, particularly in species and tissues that do not represent the virus's natural permissive hosts. Indeed, polyomavirus means "many tumor virus."
Population genetics	Interdisciplinary field of evolutionary biology, genetics, and ecology, concerned with the origins, maintenance, evolution, and significance of genetic diversity within and between populations and closely related species.
Reactivation	The reinitiation of viral replication in its host cell after a period of latency by the virus.

Table 1
Continued.

Key concept	Brief definition
SV40	Simian virus 40, which remains the general reference for all polyomaviruses. Indeed, SV40 may be the most extensively studied of all DNA viruses due to its conveniently small genome and its many historical and current uses in genetic engineering, molecular biology, and cancer research. Monkeys are the natural permissive host for SV40. This virus was first isolated from contaminated samples of polio vaccine, which were prepared from monkey kidney cells and were unknowingly given to human patients. Although SV40 has been associated with tumors in rodents and human tissue cultures, no clear evidence has been found for an increased frequency of cancers or other pathologies in the general population due to the virus.
Transformed cells	Cultured cells that exhibit the genetic and physical characteristics of a cancerous tumor (e.g., the loss of normal controls on cellular growth and death).
Viral protein synthesis	Protein synthesis refers to the production of the final functional protein for a gene. This synthesis begins with the transcription of the DNA sequence for the gene into its complementary messenger RNA (mRNA) sequence. It then continues with the translation of the mRNA into the amino acid sequence of the protein itself. Thus, viral protein synthesis refers to the transcription and translation of viral genes into their final functional proteins.

For further introductory information, the authors recommend *Basic Virology* [12] and *Fields' Virology* [13] for the virology concepts, *Evolution* [39] for the evolution terms, and *Genes IX* [40] for those related to molecular biology.

(polyomaviruses [mesh] OR polyomaviridae [tw] OR papovavirus* [tw] OR papovaviridae [mesh]) AND (virus integration [mesh] OR integrat* [tw] OR nonintegrat* [tw] OR non-integrat* [tw]) AND humans[mesh]

Again, you retrieve over 200 articles. By reading the titles and abstracts, you note that the majority of the papers are about JCV's relative, SV40, and/or about cancers or gene therapy. However, the abstract of the paper by Lednicky and Butel [27] promises a review of the biology of the polyomaviruses and, as such, may well include information on JCV. The paper by Doerfler looks intriguing, as the title refers specifically to integration of viral DNA, and the paper is indexed with the terms ''polyomavirus'' as well as ''simian virus 40'' [28]. It is unclear, however, whether it will cover JCV; there is no abstract available. You set both of these papers aside for the biologist.

SUMMARIZING THE LITERATURE

You look first at the three papers from 1983 [23–25]. Although two of the papers [23, 24] did not specifically represent normal, human, permissive cells, their titles and abstracts are intriguing, because they specifically address nonintegration, the kidney, or normal versus diseased tissues, respectively. When you read the papers, however, it is clear that they do not provide the required information. One of the two papers [23] looked at both patients who had PML and individuals who did not have the disorder; however, no evidence of JCV was found at all in the healthy participants, making the paper useless for answering the question at hand. The other paper [24] noted that in one PML patient, most of the JCV DNA in the kidney was not found to be complexed with host DNA. Although it used two non-patients as controls for the study, the paper did not discuss the integration issue for these individuals and therefore is removed from consideration.

The Chesters et al. paper [25], however, proves to be useful. The paper is unique in that it does provide direct physical evidence of the absence of JCV integration in the kidneys of 30 normal individuals who died from different, unrelated medical conditions. Specifically, total DNA from the kidneys of these 30 individuals was isolated from their cadavers and then screened with a probe for JCV DNA via DNA/DNA hybridization with Southern* blotting [29]. In this manner, JCV DNA was detected in the DNA samples from 3 of the 30 individuals and documented as unlinked to their human host chromosomes. Thus, these experiments offer direct physical evidence of JCV DNA that is episomal rather than integrated in the kidneys of normal adult and juvenile humans. However, of importance is that JCV DNA was detected in only 10% of the 30 tested individuals, even though it is currently accepted that approximately 90% of all adults are infected with the virus

Table 2
Summarizing the individual key articles

Article	General importance	Results
Padgett et al., 1971 [2]	Described original isolation and characterization of JCV	Did not address integration
Imperiale et al., 2007 [1]	Chapter on polyomaviruses from classic textbook for virology (review rather than original research)	States that integration status of JCV in normal human host is still unknown
Chesters et al., 1983 [25]	Normal (kidney, n=29; brain, n=22) and diseased cadavers (kidney, n=1; brain, n=6) tested for JCV integration with Southern hybridization technique	No integration observed in individuals; caveat: found virus in far fewer individuals than would be expected in general population; not cited in later works related to integration
Lednicky and Butel, 1999 [27]	Review of the biology of polyomaviruses, including JCV	Provides 2 reasons for no integration in normal humans: no integrase; rare and random integration of polyomaviruses in nonpermissive hosts; caveats: both reasons are inferential rather than experimental evidence
Khalili et al., 2006 [31]	Review of the biology of JCV, including life cycle	Provides diagram of life cycle, does not include integration in diagram or discussion; caveat: no citations or experimental evidence provided for integration question

[3]. The unexpectedly low JCV DNA prevalence of 10% that Chesters et al. observed brings up the question of whether the methods employed by these authors [25] are most appropriate for the detection of JCV in infected kidney cells in general and integrated viral DNA in particular. Of additional concern is that this paper is not cited in the section on polyomaviruses in *Fields' Virology* [1], nor is it cited concerning DNA integration in the additional articles that you find to be most useful (see below).

You move on to the volume of *Advances in Experimental Medicine and Biology* [26]. Chapter 1 includes a section on ''Life Cycle'' and notes, on pages 4 to 5, that ''BKV-DNA is integrated into the host cell genome in rodent cells, but in human cells, it may remain as free unintegrated copies'' [30]; there is no mention of either condition in JCV. You remember that BKV is a close relative of JCV and that they both normally infect humans. This may be another line of evidence that JCV remains nonintegrated in humans. You look through the table of contents and notice a chapter by Khalili on JCV [31]. The biologist had mentioned in your earlier conversation that Khalili is an expert on JCV and that Kitchen et al. [7] originally cited one of his recent papers [11] to support the absence of viral integration. You look through this newly discovered chapter and find that the life cycle of JCV is clearly presented in Figure 1, with no indication that JCV normally integrates into its human host chromosomes. Although this is not explicitly stated, the lack of discussion of integration or its depiction in the figure suggest that JCV does not normally integrate.

You turn now to the Lednicky and Butel paper [27], which does summarize the biology of JCV, along with other polyomaviruses such as BKV and SV40. Of all the references you have found, the strongest statement about normal JCV integration is made in this review on page 156: ''Thus, integration into the host chromosome is an incidental event and not a normal part of the polyomavirus life cycle.'' This explicit statement against normal integration is presented based on two general facts about polyomaviruses: (1) that they lack the integrase* gene for integration and (2) that integration occurs only rarely and randomly in their nonpermissive hosts. Thus, even here, no direct experimental evidence is provided about normal JCV integration.

Finally, your perusal of papers from your last search leads you to an earlier review by Doerfler [28], who summarizes different experimental studies of viral integration in both permissive and nonpermissive hosts. Thus, Doerfler's review and citations interest you, because they discuss experimental evidence for integration by polyomaviruses (but not JCV) in both permissive and nonpermissive hosts. You note earlier works cited by this author that provide experimental results for SV40 integration in permissive African green monkeys, as determined by Southern hybridization (as used by Chester et al. [25]). However, in the text, the author challenges on technical grounds the validity of some of the evidence presented by Hirai and Defendi for SV40 integration [32]. You quickly perform a ''cited reference'' search in Web of Science to see who has cited Hirai and Defendi's challenged article [32]. From this search, you retrieve an article by Rigby and Berg [33] that provides evidence that at least one of the assertions presented by Hirai and Defendi is incorrect. Rigby and Berg deduce that large pieces of DNA that Hirai and Defendi interpret as SV40 DNA integrated with human DNA are in fact, concatenated SV40 DNA—individual SV40 DNA molecules linked together. In addition, you remember from your general readings about SV40 that African green monkeys are not the natural normal host for SV40, even though they are a permissive one. Rather, rhesus monkeys are the natural host for SV40 in the wild. Given this information, you decide to rely on the data you specifically have for JCV, rather than extrapolating from its relative SV40. Table 2 includes brief summaries of the key items you include in your final selection for the biologist.

CONCLUDING REMARKS

Of the JCV articles you have identified, none provides definitive experimental evidence for or against viral integration in the kidneys of normal adult and juvenile humans. One explanation for the dearth of such ideal articles is that JCV is difficult to grow in the laboratory due to its very narrow host range in tissue culture. This fact becomes apparent when you read an additional article from the *Advances in Experimental Biology and Medicine* volume on polyomaviruses retrieved by one of your searches [34]. JCV can be grown in human embryonic kidney cells but not efficiently and not in tissue cultures from adults. This inability to efficiently grow JCV makes it very difficult to experiment with the virus under the controlled conditions of the laboratory, which are necessary for the precise tests and demonstrations of whether or not it normally integrates in the kidneys of adult and juvenile humans.

You inform the biologist that the primary literature is at least consistent with the original statement of Kitchen et al. [7], noting no non-refuted indication of normal JCV integration in the literature. As such, JCV remains separate from its host chromosomes during its normal life cycle and therefore does not evolve at the same slow rate as that of human chromosomal DNA. Correspondingly, your results support the final conclusion of a rapid evolutionary rate* for JCV as reported by Kitchen et al. [7].

The biologist emphasizes the critical importance of your involvement in this research, given that the evidence for lack of JCV integration is supportive rather than definitive. Your coauthor stresses that the existence of even just one or two articles with strong, direct, experimental evidence of normal viral integration would be sufficient to challenge the current acceptance of no integration and therefore the conclusion of Kitchen et al. [7] of a fast JCV rate. Your expert searches and reviews allow for a much greater degree of certainty that no such contradictory articles with direct compelling evidence currently exist in the primary literature. Your collaboration thus supports a great increase in the researcher's confidence and is acknowledged as an invaluable contribution to his knowledge on the topic, confirming the appropriateness of the position taken by Kitchen et al. [7].

ACKNOWLEDGMENTS

The authors thank James M. Pipas, University of Pittsburgh, for his discussion with them during his visit to the University of Florida. The authors also thank Andrew Kitchen for his literature searches in support of Kitchen et al. [7] and Christopher Brecht for his assistance in preparing the manuscript.

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